IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Zheng Xin Dong *et al.*	Examiner : WEGERT, Sandra L.
Serial No. : 09/674,597	Art Unit : 1647
Filed : April 9, 2001	
Title : PTH2 RECEPTOR SELECTIVE COMPOUNDS	

Commissioner for Patents
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DECLARATION OF DR. MICHAEL CHOREV
UNDER 37 C.F.R. §1.132

I, Dr. Michael Chorev, hereby declare and state that:

1. I am familiar with the subject matter claimed in the above-identified patent application, U.S. Serial No. 09/674,597, which is a national phase application based on International Application No. PCT/US99/09521 [WO 99/57139].

2. I have a Ph.D. in Chemistry and I currently serve as Associate Professor at Laboratory for Translation Research, Harvard Medical School (hereinafter "Harvard"), One Kendall Square, Building 600, Rm 348, MA 02139.

3. I have performed, or instructed other employees, associates or consultants of Harvard, to synthesize, purify and characterize, as described in U.S. Serial No. 09/674,597, the following four PTH analogues:

   I. [Cha^{7,11}, des-Met^{8}, Nle^{18}, Tyr^{34}]hPTH(1-34)NH_2

   II. [Cha^{7,11}, D-Nle^{8}, des-Met^{18}, Tyr^{34}]hPTH(1-34)NH_2

   III. [Cha^{7,11}, D-Nle^{8}, Nle^{18}, Tyr^{34}]hPTH(1-34)NH_2

   IV. [Cha^{7,11}, D-Nle^{8,18}, Tyr^{34}]hPTH(1-34)NH_2

4. The PTH analogues were evaluated for receptor binding affinity in the osteosarcoma cell line Saos-2/B-10, which endogenously expresses the PTH/PTHrP receptor (~20,000 receptors/cell), and BP-16 cells, which are HEK-293 cells stably expressing recombinant human PTH2 receptor (~160,000 receptors/cell). Currently, there is no cell line which endogenously expresses the PTH2 receptor. Both PTH(1-34) and PTHrP(1-34) display high affinities (IC_{50} = 3.2 and 4.0 nM, respectively) and completely inhibit binding of the radioligand ^{125}I-PTH in Saos-2/B-10 cells. Only PTH(1-34) binds to BP-16 cells and has affinity one order of magnitude weaker than that observed in Saos-2/B-10 cells.

5. The PTH analogue with the highest affinity for PTH2 receptor was [Cha$^{7, 11}$, D-Nle^{8}, Nle^{18}, Tyr^{34}]hPTH(1-34)NH_2 (analogue III) ($IC_{50}^{PTH/PTHrP\ Rc}$ = 800 nM). This analogue was also quite selective toward the PTH2 receptor ($IC_{50}^{PTH2\ Rc}$ = 24 nM; $IC_{50}^{PTH/PTHrP\ Rc}$/ $IC_{50}^{PTH2\ Rc}$ = 33). Among analogs I-IV, the highest affinity toward the PTH/PTHrP receptor belongs to [Cha$^{7, 11}$, D-Nle$^{8, 18}$, Tyr^{34}]hPTH(1-34)NH_2 (analogue IV) (IC_{50} = 6.4 nM) which differs from analogue III only by a single Nle^{18} to D-Nle substitution. Compared to analogue III, analogue IV has 125 fold higher affinity for the PTH/PTHrP receptor and only 2 fold lower affinity for the PTH2 receptor.

6. To exclude the possibility that the observed PTH2 receptor-specific binding of analogues I-III is not related to differences in numbers of receptors expressed (~160,000 PTH2 receptors/BP-16 cell versus ~20,000 PTH/PTHrP receptors/Saos-2/B-10 cell), the binding of these ligands to C21 cells, which stably express ~400,000 PTH/PTHrP receptors per cell, was examined. As in Saos-2/B-10 cells, analogues I-III showed no significant binding to C21 cells (IC_{50} = 1000, 1000, 340 nM, respectively).

7. Both PTH(1-34) and PTHrP(1-34) are equipotent in stimulating adenylyl cyclase in Saos-2/B-10 cells expressing PTH/PTHrP receptors (EC_{50} = 3.2 and 4.0 nM, respectively). Only PTH(1-34) stimulates adenylyl cyclase in BP-16 cells (EC_{50} = 32 nM).

8. Of the analogues in this study, only [Cha$^{7, 11}$, D-Nle$^{8, 18}$, Tyr^{34}]hPTH(1-34)NH_2 (IV) has high efficacy when interacting with the PTH/PTHrP receptor (EC_{50} = 0.7 nM),

comparable to PTH(1-34) and PTHrP(1-34), stimulation of adenylyl cyclase parallels binding affinity. Despite low binding affinity to cells expressing the PTH2 receptor, analogue IV is devoid of adenylyl cyclase stimulating activity.

9. Signaling through the cytosolic calcium pathway in BP-16 cells was assayed by the stimulation of $[Ca^{2+}]_i$ transients by 10^{-7} M PTHrP(1-34), PTH(1-34) and analogues. Analogues I-III, which bind to the PTH2 receptor, but are devoid of adenylyl cyclase stimulating activity, are able to produce significant increases in $[Ca^{2+}]_i$. These analogues do not show any detectable second messenger activation in HEK/C21 cells stably expressing the hPTH/PTHrP receptor. Therefore, these analogues specifically bind to the PTH2 receptor and stimulate only the cytosolic calcium secondary messenger pathway.

10. I further declare that all statement made herein of my own knowledge are true and that statements made upon information and belief are believed to be true and further that false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

Date: 6/24/07

Michael Chorev, Ph.D.